eppendorf

BIO NEWS

No. 35 – 2011

Silence | Speed | Simplicity®: Centrifuge 5424 R

- New Box System for epT.I.P.S.® Motion
- 384 White Wells – for Your Real-Time PCR
- Eppendorf Honors Young Scientists


The Influence of UV Absorbing Substances Released from Plastic Containers (Leachables) on Photometric Analyses

UV Absorbing Substances Released from Plastic Containers Influencing Photometric Analyses · Multiplex PCR Pathogen Detection in Whole Blood · Automated Plant DNA Purification · etc.

Dear Reader!

Quality, reliability, experience, innovation – these are words that people worldwide associate with Eppendorf. Whether a new technology, a service, or the further advancement of an existing product is concerned – every detail is geared towards the needs and requirements of our users. This approach has been crowned with success in every respect, as detailed in the annual report 2010 (p. 13).

The success story of the Eppendorf microcentrifuges continues. Just recently, our Centrifuge 5424 R was awarded the "red dot design award 2011" (p. 4-5).

Our family of consumables has new additions: new twin.tec Plates with 384 wells for real-time PCR, as well as epT.I.P.S. Motion with a new ecological box system (p. 8-9).

Eppendorf honors young scientists! On page 12 we are introducing to your the recent laureates of the Eppendorf research prizes.

Further to more information and product solutions for a wide range of tasks, as always the insert contains detailed Application Notes. Last, but not least, you will have the chance to win a set of 3 pipettes when you participate in our competition.

We hope you enjoy the read!

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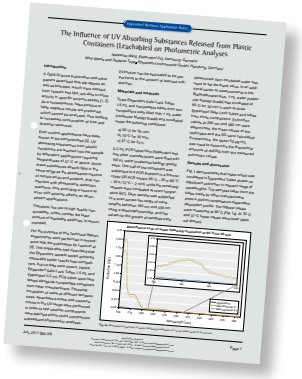


PETER SCHREINER, EPPENDORF AG

Silence | Speed | Simplicity®: Centrifuge 5424 R

The new Eppendorf microcentrifuge standard – Centrifuge 5424 R – is quieter, faster and easier to use than ever. True to our motto Silence I Speed I Simplicity, we have developed our new generation of microcentrifuges in line with the strictest requirements for operating comfort and safety. And it's paid off: launched in 2010, the Centrifuge 5424 R has now been awarded the "red dot design award 2011" as the compact 24-place microcentrifuge – the successor to the legendary Centrifuge 5415 R – offers even more comfort in the laboratory.

The Centrifuge 5424 R was developed according to comprehensive ergonomics research to meet users' constantly increasing demands. Routine tasks can be completed more quickly and easily than ever before. For example, with the FastTemp precooling function, the centrifuge achieves a time savings of 45 % compared to the previous model.

Latest generation of refrigerated microcentrifuges

The versatile compact microcentrifuge 5424 R is designed as a key element of an ideal laboratory working environment, which guarantees reliable and safe performance according to the highest standards in molecular biology, biochemistry and biotechnology.

With its compact footprint and low access height, the Centrifuge 5424 R is ideal for every lab! It comes standard with an aerosol-tight 24-place rotor for 1.5/2.0 mL tubes, and features three additional rotor options. Its patented compressor technology reduces vibrations, providing comprehensive protection for your samples.

And let's not forget the reliable temperature accuracy: the preset temperature of 4 °C remains constant even at a maximum speed of up to 21,130 x *g* or 15,000 1/min.

Prize-winning!

The design and unique ergonomics of the Centrifuge 5424 R have now been honored. Subject to the strictest evaluation criteria, which include degree of innovation, functionality, longevity and ecological requirements, the Centrifuge 5424 R has proven its merit with the awarding of the red dot design award 2011.

reddot design award
winner 2011

Awarded annually since 1955, the red dot award recognizes global design achievements for a wide variety of industrial products.

Silence I Speed I Simplicity®

The Centrifuge 5424 R doesn't just stand out with its excellent ergonomics – it perfectly embodies our motto Silence I Speed I Simplicity. It is even quieter, faster and easier to operate than its predecessor.

Silence! Quiet for a comfortable working environment

All mechanical components have been carefully selected and optimally combined to create a device with significantly reduced operating noise.

The result: the Centrifuge 5424 R is incredibly quiet even during operation without rotor lid.

Centrifuge 5424 R with keypad

The rotor chamber's new OptiBowl design markedly reduces background noise to ensure a pleasant working environment.

Speed! Faster and more flexible for all applications

The powerful Centrifuge 5424 R offers a high centrifugation speed of up to 21,130 x *g* (15,000 1/min), in a temperature range of -10 °C to +40 °C. Quick precooling, e.g., from 21 °C to 4 °C, can be achieved in just 8 minutes with the FastTemp function. Moreover, a secure SOFT brake provides maximum protection of sensitive samples.

Simplicity! Safety via intuitive operation

For Eppendorf, intuitive operation means fast and safe handling without time-consuming training. Because routine processes frequently need to be mastered quickly and easily to guarantee a high level of safety in performance.Therefore, the Centrifuge 5424 R is equipped with a display which is easy to read and operate. The device is also available in two versions: with rotary knobs for quick parameter setting or an easy to clean keypad.

Another plus: flexible rotor selection

The Centrifuge 5424 R can be used with four different rotors according to individual needs.

- Aerosol-tight rotor for 24 x 1.5/2.0 mL tubes
- Kit rotor: for safe centrifugation of 18 x spin columns or 1.5/2.0 mL tubes with open tube lids. The raised edge of the Kit rotor prevents open tube lids from breaking off during centrifugation.
- Aerosol-tight rotor (for 24 x 1.5/2.0 mL tubes) with PTFE coating for increased chemical resistance
- 4 x PCR strip rotor

Kit rotor™ (F-45-18-11-Kit)

Our contribution to the environment: epGreen

Eppendorf demonstrates its commitment to a green planet with the epGreen initiative, whose objective is to constantly reduce the environmental impact of our business operations and products. Therefore, the Centrifuge 5424 R was developed according to the latest emissions and environmental requirements while also setting new standards in these areas.

The baseline power consumption has been reduced by 25 % compared to the previous model. The centrifuge saves up to 60 % in power consumption with the FastTemp function for quick precooling.

epGreen®

Would you like additional information?

The Centrifuge 5424 R brochure can be ordered from us using the ref. no. denoted below.

Detailed information is also available at www.eppendorf.com/centrifugation.

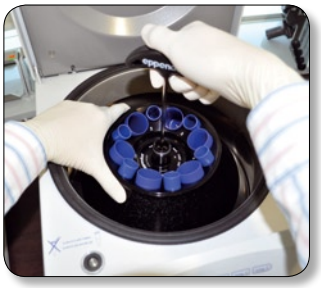
Centrifuge 5424 R • Ref. no. 235

JAN-HENDRIK BEBERMEIER, EPPENDORF AG

Safe and Efficient Centrifugation

Do you already know the new Eppendorf poster "Maintenance of Centrifuges"? It provides you with useful advice on the proper cleaning and maintenance of your Eppendorf centrifuge, as well as instructions for the correct rotor loading and other interesting and smart tips for safe and efficient centrifugation. Here are some examples from the contents:

Fasten rotor tightly



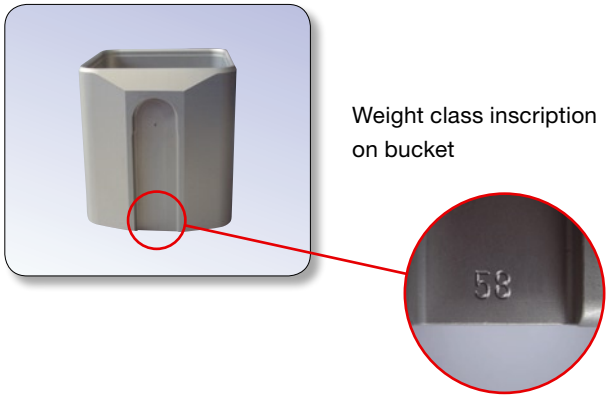
Prior to centrifugation, rotor must be tightened securely on drive shaft using a rotor key. For swing-bucket rotors, ensure that buckets are properly hooked onto the rotor. Perform a manual swing-out test to check that the buckets are moving freely.

Consider max. capacity

Note the weight specifications printed on the rotor. Example: 4 × 1.1 kg means weights of each bucket + adapter + tubes filled with sample must not exceed 1.1 kg. Take note of the maximum g-force specified for the tubes you are using.

Apply correct buckets

In swing-bucket rotors buckets with the same weight class must be on opposite positions. To check the weight category of the bucket, check the value at the side of the buckets.



Weight class inscription on bucket

Choose correct adapter

Adapters must support tubes securely. The tube should fit tightly into the adapter.

wrong

Wrong use of adapter:
no secure support of upper part

right

Correct use of adapter

For conical tubes use conical tube adapter with conical base.

For tubes with round bottom use tube adapter with flat base and rubber mat.

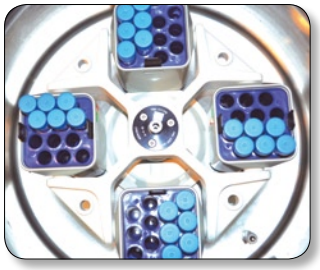
Rotor loading

wrong

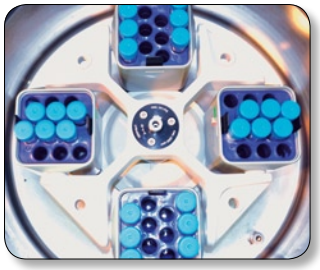
right

Fixed-angle rotors:

- Load symmetrically and balance weights.



wrong



right

Swing-bucket rotors:

- Bucket and adapter loading must be symmetric and balanced.
- Position tubes in buckets in such a way that rotor pivots are stressed evenly.
- Always have all 4 swing buckets on rotor even though only 2 might be in use.

The DIN A2 Poster “Maintenance of Centrifuges” (Order no. AQ18636020) is an ideal complement to your manual.

If you wish to decorate your lab wall with it, please ask your Eppendorf sales rep for a personal copy (free of charge).

KAY KÖRNER, EPPENDORF AG

384 White Wells – for Your Real-Time PCR

The new Eppendorf twin.tec 384 real-time PCR plates combine all the advantages of the normal twin.tec plates with 384 white wells for your real-time PCR.

New! Eppendorf twin.tec® 384 real-time PCR plates

The white wells reflect fluorescence much better than clear wells, enabling the measurement of samples with lower fluorescence intensity. In each single well! White wells also greatly reduce interfering background fluorescence, leading to improved homogeneity of replicates and increased reproducibility of results.

New! Masterclear® real-time PCR film

New! Masterclear® real-time PCR Film

We recommend Masterclear real-time PCR Film, adhesive, as the ideal sealing option for your real-time PCR plates.

- High light transmission (> 90 % between 450 nm and 750 nm) for sensitive real-time PCR
- Effective adhesive sealing prevents evaporation during real-time PCR
- Residue-free removal from the plate
- PCR clean = certified free of human DNA, DNase, RNase and PCR inhibitors

More information

Learn all there is to know about Eppendorf PCR plates at www.eppendorf.com/pcrplates or request the latest brochure on real-time PCR consumables, "The bright choice", using the reference number denoted below.

"The bright choice" brochure • Ref. no. 223

News

Interference with DNA Measurements by Plastic Consumables

It is nearly impossible to imagine our daily life without synthetic materials. In molecular and biochemical laboratories, consumables made from plastic are used in practically every application.

However, in the recent past, scientific reports of skewed DNA measurement results due to certain leachable additives, which are used during production by some manufacturers of plastic consumables, have accumulated. Not only sensitive enzymatic assays are affected. As described in the Application Note on p. 1–2, simple heating of the sample in plastic containers made by certain manufacturers can lead to leaching of substances which critically interfere with the detection of biomolecules such as DNA and proteins, and which may thus skew concentration measurements.

Warming of samples occurs during many routine applications in molecular or cell biology, such as cell lysis, centrifugation or PCR. By avoiding slip agents, biocides, plasticizers and other substances which may interfere with DNA measurements, Eppendorf tubes and tips are ideally suited for all sensitive detection methods in the laboratory (www.eppendorf.com/consumables).

Therefore, rely on Eppendorf quality for reproducible and reliable results!

DANIEL WEHRHAHN, EPPENDORF AG

The Influence of UV Absorbing Substances Released from Plastic Containers (Leachables) on Photometric Analyses

Natascha Weiß, Eppendorf AG, Hamburg, Germany
Wolf Wente and Stefanie Topp, Eppendorf Instrumente GmbH, Hamburg, Germany

Introduction

A 2008 *Science* publication and other papers described that slip agents as well as biocides, which were washed from vessels and tips, are able to show activity in specific enzyme assays [1, 2]. As a consequence, false positive or false negative results are produced, which cannot be analyzed, thus leading to increased consumption of time and financial resources.

Even routine applications have been shown to be compromised [3]. UV absorbing substances from plastic containers are leached into the sample by laboratory applications requiring temperatures of 37 °C or above. Since these substances absorb light in the same range as the absorbance maxima of nucleic acids and proteins, they can interfere with photometric detection reactions, thus providing a source of error with adverse effects on downstream applications.

Therefore, the use of high quality consumables, which contain the least amount of leachable additives, is recommended.

For the purpose of this Technical Report, experiments were performed in accordance with the publication by Lewis *et al.* [3]. This publication had described that the Eppendorf vessels tested achieved noticeably better results than competitors, but no data were shown. Hence, Eppendorf Safe-Lock Tubes 1.5 mL and Eppendorf 0.2 mL PCR tubes were here tested alongside comparable containers from other manufacturers. Following incubation of water at different temperatures, absorbance scans and measurements in the UV range were performed in order to test whether components were leached which could compromise subsequent photometric analyses. Extinction can be estimated to be proportional to the amount of leached substances.

Materials and methods

Three Eppendorf Safe-Lock Tubes 1.5 mL and comparable tubes from two competitors were filled with 1 mL water (molecular biology grade) and incubated under the following conditions:

a) 95 °C for 30 min,
b) 70 °C for 30 min,
c) 37 °C for 24 h.

0.2 mL PCR tubes from Eppendorf and two other manufacturers were filled with 150 µL water (molecular biology grade) each. One half of the containers was subjected to a PCR protocol in a thermocycler (30 PCR cycles: 95 °C – 30 s/60 °C – 30 s/72 °C – 2 min), while the remaining vessels were incubated at room temperature (RT). Each sample was subjected to a scan across the range of wavelengths between 190 nm and 400 nm using a spectrophotometer, and the values for the groups of samples were determined. Non-incubated water was used to set the blank value. In an additional series of measurements in the BioPhotometer plus, 1 mL water (molecular biology grade) was incubated at 95 °C for 30 min in each of three Eppendorf Safe-Lock Tubes and tubes from three competitors. Extinction values at 260 nm and 280 nm were determined, the mean values of the replicates and the SD were calculated. Furthermore, the factor 50 µg/mL was used to determine the theoretical amounts of dsDNA from the measured extinction values.

Results and discussion

Fig. 1 demonstrates that water which was incubated in Eppendorf Tubes shows no significant extinction in relevant range of wavelengths. The samples taken from the tubes made by other manufacturers show a distinct temperature-dependent absorption profile. The highest values were measured at 95 °C (Fig. 1a). At 70 °C and 37 °C these values were lower (data not shown).

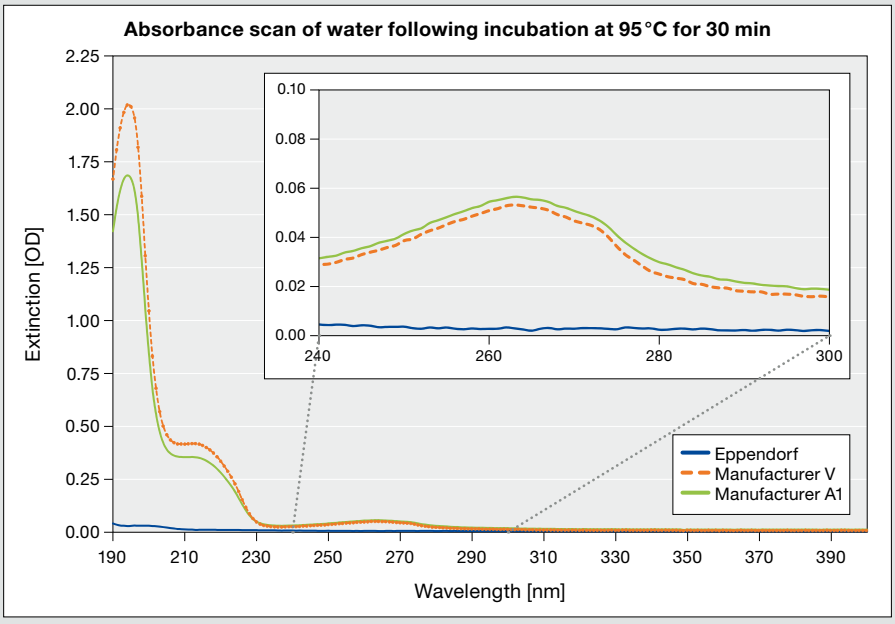


Fig. 1a: *Absorbance spectrum of water following incubation of 1.5 mL tubes at 95 °C for 30 min*

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The Influence of UV Absorbing Substances Released from Plastic Containers (Leachables) on Photometric Analyses

Comparable results were obtained for the PCR tubes: the water taken from competitors' tubes treated in the thermocycler yielded higher extinction values than the water incubated at room temperature (Fig. 1b).

According to these data, it appears that substances which absorb light in the UV range are released from the competitors' containers tested herein. The maximum values are observed slightly above 190 nm. In addition, water from the 1.5 mL tubes showed absorption signals in the range of 210-220 nm as well as 260 nm (Fig. 1a).

It is exactly this set of wavelengths which plays an important role during photometric detection and quantification of biomolecules. For example, proteins may be measured directly at 205 nm or at 280 nm, whereas nucleic acids are measured at 260 nm. One consequence of leached UV absorbing components is illustrated in Fig. 2: Here, extinction values of water incubated at 95 °C in a further experiment are shown at 260 nm and 280 nm. In addition, the theoretical amount of dsDNA, which would result from this value, is shown. The value for the samples obtained from the Eppendorf Safe-Lock Tubes was below the detection level (LOD = limit of detection), whereas the water from the other tested vessels yielded values above the limit of quantification (LOQ). Therefore, elevated results for DNA concentration would be obtained during DNA quantification. When the actual amount of DNA present is lower than the measured value, a negative impact on downstream applications may ensue.

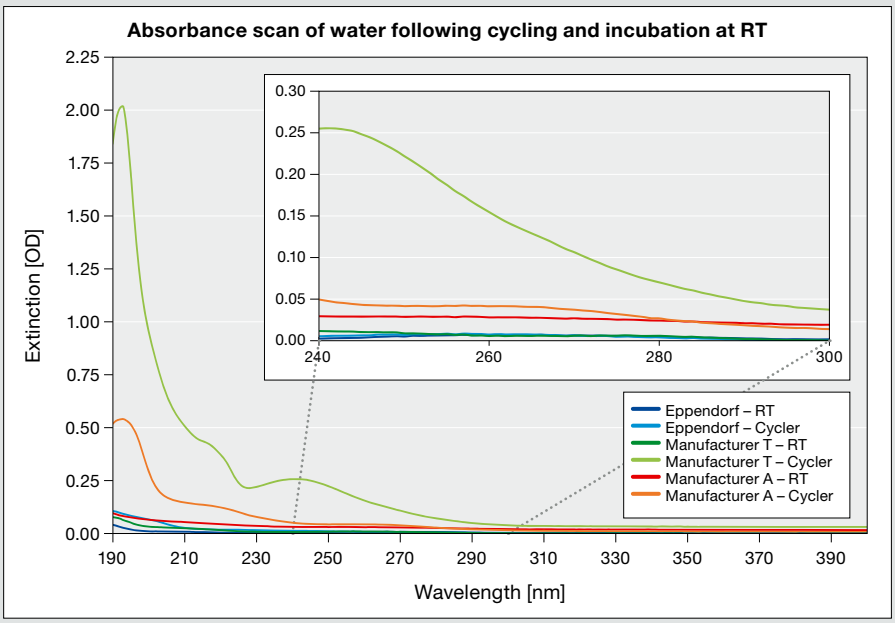

Fig. 1b: *Absorbance spectrum of water following incubation of 0.2 mL PCR tubes in the cycler and at room temperature (RT)*

Conclusion

Photometric analyses verified that UV absorbing substances are released from plastic vessels made by certain manufacturers, and that they interfere with the detection of biomolecules such as nucleic acids and proteins.

Samples treated in Eppendorf Tubes did not show significantly compromised values. Here, as in a number of published investigations based on sensitive cell-based assays, differences between plastic consumables by different suppliers became evident. By omitting lubricants and other additives during production, Eppendorf tubes are very well suited for sensitive detection methods in the laboratory.

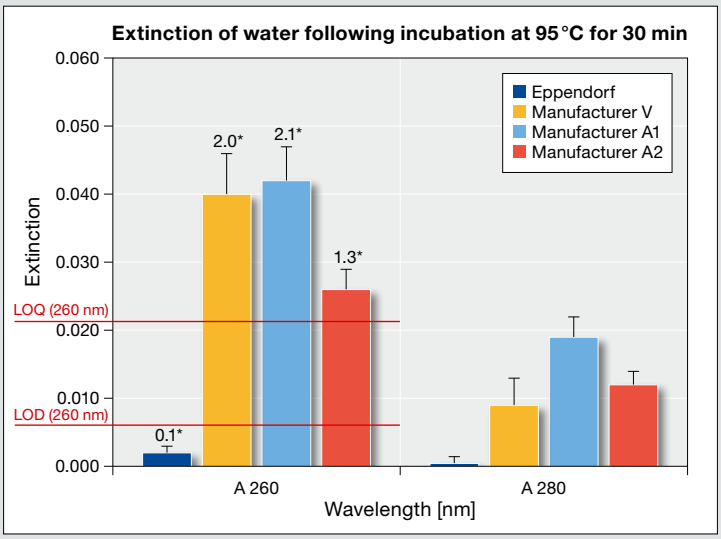

Fig. 2: *Extinction of water at 260 nm and 280 nm following incubation in 1.5 mL tubes at 95 °C for 30 min*

**Values displayed represent the "theoretical" amount of dsDNA [µg/mL] calculated from the extinction values.*

The resulting LOD (limit of detection) was determined at 0.006 E ≙ 0.3 µg/mL; the LOQ (limit of quantification) was determined at 0.021 E ≙ 1.0 µg/mL.

Literature

[1] McDonald GR, Hudson AL, Dunn SM, You H, Baker GB, Whittal RM, Martin JW, Jha A, Edmondson DE, Holt A. Bioactive contaminants leach from disposable laboratory plasticware. *Science* 2008; 322(5903):917

[2] McDonald GR, Kozuska JL, Holt A. Bioactive Leachates from Lab Plastics. *G.I.T. Laboratory Journal Europe* 2009; 13:24-26

[3] Lewis LK, Robson M, Vecherkina Y, Ji C, Beall G. Interference with spectrophotometric analysis of nucleic acids and proteins by leaching of chemicals from plastic tubes. *BioTechniques* 2010; 48(4) 297-302

More on Eppendorf Safe-Lock Tubes at www.eppendorf.com/consumables

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Removal of Fluorescently-labeled Sensory-neurosecretory Cells from Forebrain of Transgenic *Medaka* Embryos Using Eppendorf PatchMan™ NP 2 and CellTram® vario and Rowiak CellSurgeon

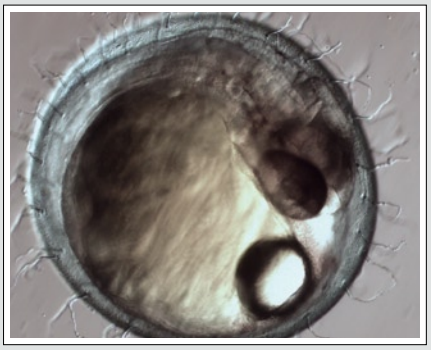
Kristin Tessmar-Raible[1], Katharina Schipany[1] and Sabine Przemeck[2]
[1]Max F. Perutz Laboratories, University of Vienna, Austria
[2]Rowiak GmbH, Hanover, Germany

Abstract

For a representative analysis of cell clusters in transgenic small animal models, cell material with the least possible contamination by nondescript cells is desirable. Here we report the successful removal of fluorescently-labeled sensory-neurosecretory cells from the forebrain of transgenic Medaka embryos combining laser-assisted excision (CellSurgeon, Rowiak GmbH) and extraction of excised cells using CellTram vario and Eppendorf PatchMan NP 2.

Introduction

Medaka is a well-established model organism for the analysis of cell signalling in developmental and neurobiological research [1].



Transgenic animals expressing fluorescent proteins under specific enhancer/promoters are of special interest as they provide a tool to obtain a better understanding of control mechanisms during different stages of development. In this context, the analysis of the impact of environmental factors on the cell's transcriptome is of great interest.

Such transcriptome analyses can be performed by modern technologies including next generation sequencing. In order to be successful, starting material that is as "pure" as possible is required. However, gaining cell material of such quality from small pieces of tissue, such as a *Medaka* forebrain, is a challenging task. We approached this challenge by using Rowiak CellSurgeon in combination with an Eppendorf micromanipulator set up. Rowiak CellSurgeon is a laser scanning microscope with cutting ability, which was used to image, measure and excise cell clusters of sensory-neurosecretory cells expressing m-cherry red from the forebrain of *Medaka* embryos. Excised cell clusters were then removed with a 10 µm microcapillary controlled by PatchMan NP 2 micromanipulator and CellTram vario microinjector.

Material and methods

Micromanipulation set up

- PatchMan NP 2 (Eppendorf)
- CellTram vario (Eppendorf)
- CustomTip Type I: 10 µm, blunt end capillaries (Eppendorf)

CellSurgeon (Rowiak, Hanover, Germany) equipped with an

- Inverted microscope AxioObserver D1 (Zeiss, Jena, Germany)
- T pulse 200: 10 MHz; 2.5 W, 1030 nm (Amplitude Systems, Pessac, France)
- Camera (Jenoptik, Jena, Germany)

Animals

- *Medaka* embryos (line 2216) with m-cherry red labeled sensory-neurosecretory cells (K. Tessmar-Raible)

Other material

- 35 mm Petri dish with 0.17 mm glas bottom (WillCo Wells, Amsterdam, Netherlands)
- 6 % methyl cellulose
- Embryo rearing medium (1x ERM)
- 4 % Tricaine methanesulfonate (stock solution)

Preparation of 35 mm dishes and adjustment of micromanipulation set up

In order to access the forebrain in the flattest angle possible dishes were cut down to a height of 4 to 5 mm and the capillary holder was inserted in the lower mount on the X head. The angle for the universal capillary holder was adjusted in such a way that is was possible to move the capillary directly along the glass bottom until it reached the embedded embryo on the same focal level. This was necessary in order to avoid shifting the position of the embryo once cell clusters had been identified since fine movements of the microcapillary were considerably hampered and slowed by the viscous methyl cellulose.

Mounting of embryos

Chorionated embryos were anaesthetised with 0.4 % Tricaine and mounted onto the glass bottom of a 35 mm dish partially filled with 6 % methyl cellulose. Once embryos had been carefully orientated with eyes facing the glass bottom they were screened for fluorescent cell clusters (Fig. 1).

Embryos that had been screened positively were sacrificed with an overdose of Tricaine methansulfonate and manually hatched under a stereomicroscope. Hatched/dechorionated embryos were also mounted using 6 % methyl cellulose, but placed on their backs with heads close to the dish bottom.

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Removal of Fluorescently-labeled Sensory-neurosecretory Cells from Forebrain of Transgenic *Medaka* Embryos Using Eppendorf PatchMan™ NP 2 and CellTram® vario and Rowiak CellSurgeon

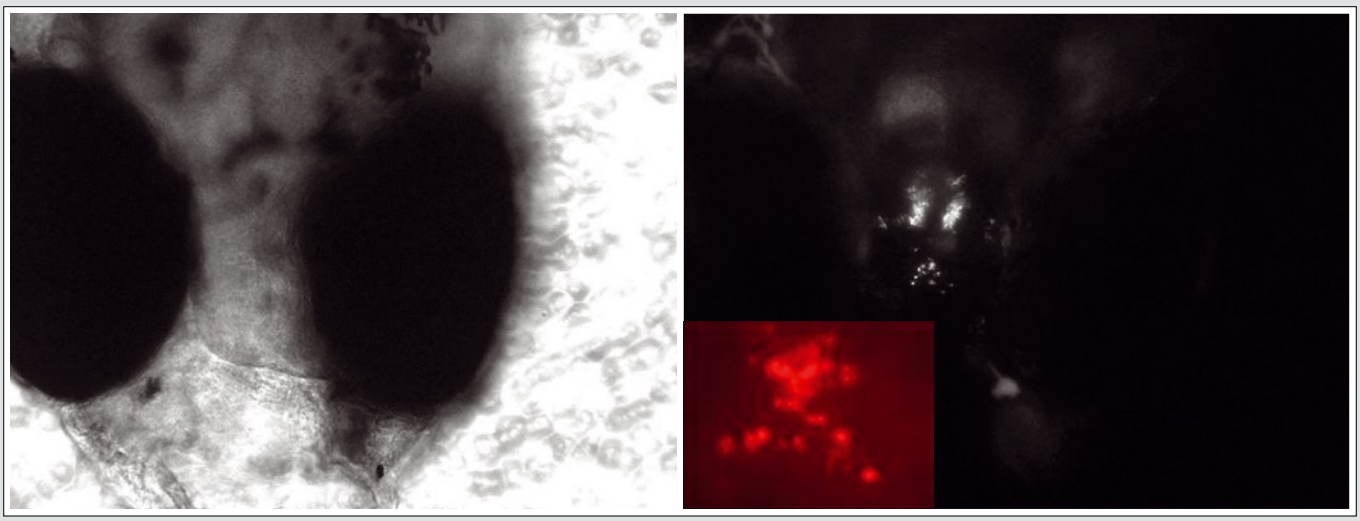

Fig. 1

Positioning of microcapillary

Using 20x magnification and brightfield imaging the microcapillary was brought into focal plane in close proximity of the embryo's head. Magnification was changed to 40x for precise positioning and rechecked using fluorescence imaging to establish an exact position of tip in comparison to cell cluster. This position was stored as position 1 using the PatchMan NP 2. The microcapillary was withdrawn a few millimeters and then slightly moved upwards.

Laser-assisted excision of cell clusters

Cell clusters were imaged in laser scanning mode at 40x magnification to determine their x-, y- and z-dimensions. The parameters established were then used to excise cell clusters in cubical fashion and concomitantly to minimize contamination with unlabeled cells by choosing cutting lines as close as possible to cell clusters (see Fig. 2).

Removal of cell clusters

As shown in Fig. 3, excised cell clusters were imaged in fluorescence mode at 20x or 40x magnification and the microcapillary was slowly (fine or extra fine mode) brought back to position 1.

Cell clusters were aspirated using the fine drive of the CellTram vario. The microcapillary was slowly withdrawn and then moved to the Home position. Cell clusters were transferred into a microcentrifuge tube turning the dial for fine drive clockwise.

Results and discussion

With the use of laser-scanning microscopy (Rowiak Cell Surgeon) we were able to determine dimensions of cell clusters in order to excise those in cubical fashion. Excised cell clusters were made up of an estimated 75 % of cells of interest, so that further molecular biological analysis such as transcriptome analysis would be possible. The high resolution of microsteps offered by the PatchMan NP 2 allowed precise positioning of the microcapillary before as well as after excision.

Successful removal of cell clusters was feasible as CellTram vario offers a very small minimum adjustment volume (fine drive) and therefore allows to generate a vacuum just right to remove cells, but without detaching single cells from clusters.

The above described method could be of great value to researchers working with transgenic small model organisms trying to unravel genetic traits.

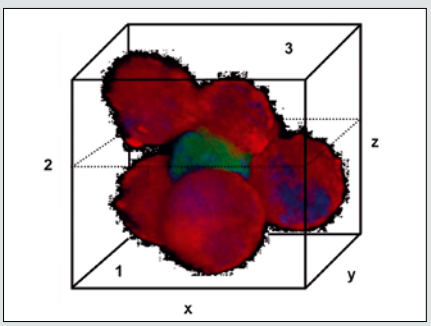

Fig. 2

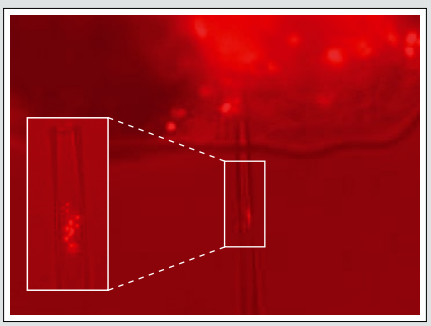

Fig. 3

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Medaka – a model organism from the Far East.
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Rapid and Reliable Multiplex PCR Pathogen Detection in Whole Blood by Using the VYOO® Kit on the Mastercycler® pro

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Abstract

Here we introduce a new protocol for multiplex PCR-based detection and identification (VYOO, SIRS-Lab, Jena, Germany) of bacterial and fungal pathogens directly from whole blood. As sepsis is one of the most common causes of death in hospitalized patients a rapid pathogen detection is a cornerstone in effective therapy.

To investigate DNA isolates from whole blood samples of intensive care unit patients for the presence of sepsis-causing pathogens a microbial DNA enrichment was performed followed by a standard multiplex PCR on the Mastercycler pro.

Gel-based amplicon analysis confirmed successful nucleic acid trace detection of 34 bacterial and 6 fungal targets as well as 5 antibiotic resistances with an overall sensitivity of 10 to 100 colony forming units (cfu)/mL whole blood.

Introduction

Sepsis results from the host's response to bacterial and fungal infections, whereas malfunction of the defense and repair system seems to be responsible for the development of organ dysfunctions.

In Germany, 154,000 patients suffer from severe sepsis each year. With 60,000 deaths, it is one of the most frequent causes of death in the ICUs. About 30 % of the intensive medicine budgets are expended for the treatment of those patients [1]. A prompt and adequate antibiosis, started in the first few hours of infection, assigns the crucial step for an effective therapy [2–4]. Epidemiological data confirm that a doubling of mortality is the consequence of inadequate therapies [5] and an increase of mortality of more than 7 % per hour is proven in cases of delayed adequate antibiotic treatment [6]. In addition, development and spread of antibiotic resistances are serious health risks, which are mainly contributed to antibiotics overuse [7].

Compared to the so far routinely used culture-based methods, nucleic acid amplification techniques (NAT) allow a more rapid species and resistance detection within several hours. Possible impact on sensitivity caused by factors like e.g. salts and blood ingredients, can be abolished by affinity chromatography sample preparation. VYOO (CE-marked 2008) combines such a sample preparation with sensitive multiplex PCR-based pathogen detection and a time-to-result of less than one working day [8, 9].

Materials and methods

Mechanical cell lysis

EDTA whole blood was homogenized and poured into tube containing glass-bead matrix, antifoam solution, and *Bacillus subtilis* endospores as internal run control. Mechanical impact was applied using a FastPrep-24 cell lysis device (MP Biomedicals, Solon, OH, USA) followed by proteolytic digestion of the lysate [11].

Total genomic DNA isolation

DNA of digested lysates was isolated by applying a standard protocol and dissolved in an appropriate buffer for subsequent affinity chromatography [11].

Enrichment of bacterial and fungal DNA with LOOXSTER®

Total DNA was subjected onto affinity chromatography spin columns and after standard processing, dissolved in 30 µL water. DNA concentration was determined by photometry [11].

Multiplex PCR detection of pathogen-specific targets

Standard multiplex PCR was performed within two pools of specific primers. The assay covers 34 bacterial and 6 fungal species, including: *Bacteroides fragilis, Burkholderia cepacia, Aspergillus fumigatus, Candida albicans, Clostridium perfringens, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Klebsiella oxytoca, Neisseria meningitidis, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Stenotrophomonas maltophilia, Streptococcus agalactiae, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus sanguinis* [11].

In addition, a set of frequent antibiotic resistances (e.g. methicillin, vancomycin, and ß-lactamases) are also targeted specifically. *Bacillus subtilis*-specific primers are within each pool as internal run control. The PCR was set up using the VYOO kit PCR reaction vessels which contain the lyophilized primer mix. In addition, a PCR reaction contained 1x QIAGEN Multiplex PCR Master Mix (QIAGEN, Hilden, Germany) and ≤1 µg pathogen enriched DNA filled up with DNA-/DNase-free water. No template controls (NTC) were devoid of DNA. The PCR reaction was performed on the Mastercycler pro (Figure 1).

Multiplex PCR amplicons were analyzed on agarose gels and assigned to pathogens and/or antibiotic resistances on the basis of pool-specific length markers.

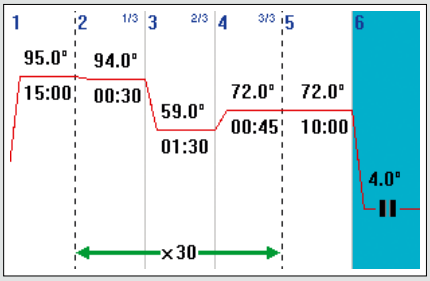

Fig. 1: *Temperature profile of the PCR program on the Mastercycler pro.*

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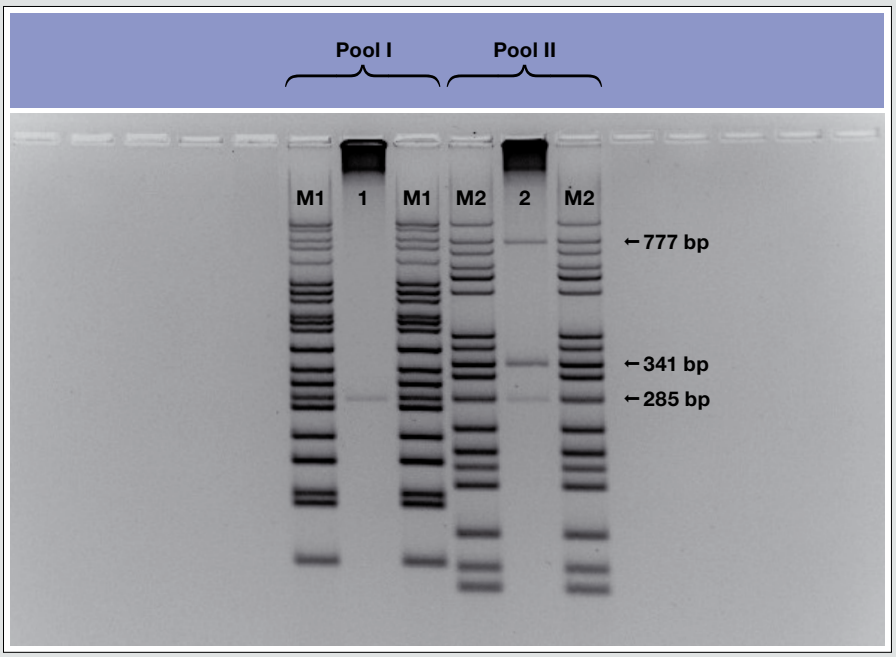

Fig. 2: *Proof of Enterococcus faecium genomic DNA in EDTA whole blood by VYOO. Samples were taken from ICU patients suspected for sepsis.* ***M1/2:*** *DNA marker pool I/II;* ***1:*** *sample DNA tested with primer pool I;* ***2:*** *sample DNA tested with primer pool II;* ***1:*** *Bacillus subtilis-specific band at 285 bp for internal run control;* ***2:*** *E. faecium-specific band at 777 bp, vanA resistance-specific band at 341 bp, B. subtilis-specific band at 285 bp (internal run control).*

Results

After preparation of exemplary EDTA whole blood samples from patients suspected for sepsis, agarose gel analysis of PCR products revealed single bands (Fig. 2). Amplicons were specific for *Enterococcus faecium* and vancomycin A resistance (pool 2) as compared to species-specific marker bands.

Positive control bands (*Bacillus subtilis*) were fully visible and the findings were confirmed by surrounding clinical results. The analytical sensitivity of the assay was tested before to be <10 colony forming units (cfu)/mL (*E. feacium* and vancomycin A resistance, respectively).

Discussion

The combination of up to 25 primer pairs in a single multiplex PCR reaction is considered to be a challenge regarding sensitivity and specificity. For pathogen detection in body fluids, e.g. sepsis-causative pathogens in whole blood, assays have to cover a capacious panel of targets to yield required clinical utility.

While broad-range primers are under ongoing discussion with respect to high numbers of false-positives and the risk of contamination [10], usage of specific primers in combination seems to be an alternative if clinical sensitivity meets the range of diagnostic relevance and if maximum specificity is given.

The presented results of the VYOO PCR detection kit indicate that nucleic acids trace detection is possible applying high plex-grades with desired sensitivity and specificity. The Mastercycler pro fulfills the demanding requirements of such a complex PCR assay: this thermal cycler helps to minimize the technical variance and contributes to a high process reliability in the PCR workflow of such a sensitive and specific assay.

Analysis of whole blood samples by multiplex PCR for bacterial and fungal DNA is a reliable and rapid tool for detection of “low level” infections. It supports and expedites clinical findings and strengthens a directed antibiotic therapy.

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[11] Further details can be taken from the Eppendorf Application Note 231 at www.eppendorf.com/applications

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Automated Plant DNA Purification Using the Nucleon® Plant DNA Kit on the ep*Motion*® 5075 TMX

Alison Hobday Botwright and Sheila Doyle, Gen-Probe Life Sciences Ltd., Manchester, United Kingdom
Eric Cairns and Warren Higgins, Gen-Probe Life Sciences Ltd., West Lothian, United Kingdom

Introduction

In the field of plant molecular biology successful analysis requires the purification of reliable and high quality genomic DNA. The purification of such DNA can be time consuming particularly in large scale projects where many individual samples need to be processed. A reliable, reproducible and easy to handle automated method for the purification of plant derived DNA is therefore very desirable.

In this report we demonstrate an automated method for the purification of plant genomic DNA using the Nucleon Plant DNA Kit from Gene-Probe Life Sciences on the Eppendorf workstation ep*Motion* 5075 TMX.

The automated method was found to deliver consistently high purity, high molecular weight DNA from the sample types tested. With a protocol time of 4 hours (with minimal hands on time) for 96 samples and no detectable cross sample contamination the methodology provides a viable easy to use automated procedure for the purification of plant derived DNA.

The Nucleon Plant DNA Kit is designed for the rapid, economical purification of high quality, high yield DNA from a wide range of plant materials. The purification process is based on the reversible binding of DNA to novel magnetic beads. The beads, which are not silica coated, have nanometre dimensions, which can aggregate to form functional complexes. These complexes provide the means to purify DNA from complex mixtures and provide consistent quality and yield.

The ep*Motion* 5075 TMX includes an integrated TMX module to shake and heat samples which can be loaded and unloaded with the gripper. The integrated TMX module means that following sample disruption the lytic process that is enhanced by incubation at higher temperatures, can be performed online and further reduces the need for hands on time. This incubation step is instrumental in delivering higher yield and purity DNA. The steps of DNA binding, washing, drying and elution are fully supported by the ep*Motion* TMX system delivering the final ready to use eluted high quality, high yield DNA for enhanced performance in downstream applications such as restriction digestion.

Materials and methods

Eppendorf epMotion TMX

Nucleon Plant DNA Kit (Gen-Probe Life Sciences, San Diego, CA, USA)

Multi Magnetic Probe Plate

Promega Magnabot 96 (Promega, Madison, WI, USA)

Sample material

Leaves of cabbage, pine, rose, lettuce and barley and popping corn. For each individual sample, 30 mg of plant material was placed into the provided sample lysis tubes containing the steel ball.

Automation

The automated procedure starts with the transfer of the sample tubes to position TMX; followed by the addition of Lysis Buffer to each of the sample lysis tubes (TMX); followed by the addition of plant neutralisation buffer. The lysis tubes are removed from the ep*Motion* and are homogenised using a mixing mill. Thereafter, the tubes are centrifuged to remove any debris and returned to the ep*Motion*.

The automated protocol continues with mixing, heating, and incubation steps. Before adding the solution of Magnetic bead & RNase, the magnetic bead suspension should be mixed carefully. The binding and washing steps are continued. The final sample is transferred to the elution plate. The total processing time is approximately 4 h for 96 samples (of which 3 h are automated processes).

Results

Yield and purity of DNA

As shown in Table 1, DNA from various plant samples can be easily purified with the Nucleon Plant DNA Kit and the automated ep*Motion* 5075 TMX system.

Sample	Initial sample weight (mg)	n	DNA Purity ($A_{260/280}$)	%CV	Mean DNA concentration (ng/µL)	Mean total yield (µg)	%CV
Cabbage	30	64	2.18	1.7	109.63	21.93	31.9
Pine	30	18	2.00	4.1	26.16	5.23	35.1
Rose	30	18	1.67	2.9	61.84	12.37	13.3
Lettuce	30	18	2.23	6.4	15.96	3.18	20.7
Corn	30	16	2.14	7.1	22.88	4.58	74.1
Barley	30	8	2.06	2.9	54.01	10.0	26.3
	Overall mean purity		**2.05**		Overall mean yield	**11.65**	

Table 1: *Yield and purity of DNA. Variability in sample yield is not unexpected when purifying DNA from plant material, this can usually be attributed to the off-line sample preparation stage.*

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Automated Plant DNA Purification Using the Nucleon® Plant DNA Kit on the ep*Motion*® 5075 TMX

The method was found to deliver consistently high purity DNA with a mean 260/280 absorbance ratio of 2.05 indicating low protein contamination in the resulting DNA samples. The average total yield across the sample types, derived from 30 mg of starting plant material, was found to be 11.65 µg.

Quality of DNA and structural integrity

In order to further demonstrate quality and structural integrity of the isolated DNA the purified DNA samples were analysed by agarose gel electrophoresis (Fig. 1).

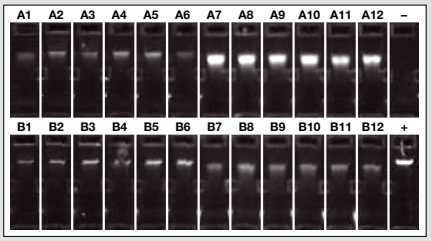

Fig. 1: *Agarose gel electrophoresis of purified DNA samples from cabbage leaves (A1–A6), pine needles (A7–A12), rose leaves (B1–B6) and corn (B7–B12). – = negative buffer control, + = Lambda DNA positive control*

The cabbage sample was further analysed with and without treatment with restriction enzyme. The automated purification procedure was shown to produce high molecular weight DNA as indicated by the clear bands (Fig. 2A). Efficient restriction enzyme digestion was achieved with the gels producing the characteristic smear of DNA (Fig. 2B).

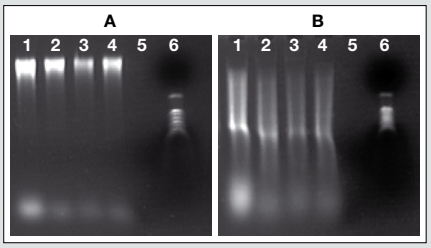

Fig. 2: *Gel electrophoresis of purified DNA samples from cabbage leaf without (A) and following restriction digestion (B).*

Lanes 1–4: purified cabbage DNA
Lane 5: negative control
Lane 6: DNA-ladder

Sample	Initial sample weight (mg)	n	DNA Purity ($A_{260/280}$)	Mean DNA concentration (ng/µL)	Mean total yield (µg)	%CV
Cabbage	30	48	2.19	104.88	20.98	33.0
Empty	0	48	0.91	1.12	0.22	–

Table 2: *Spectrophotometric assessment of cross contamination*

Cross contamination

Spectrophotometric assessment of cross contamination indicates that no DNA can be detected from the 48 cabbage leaf sample preparations in the negative control wells (Table 2).

This finding was further supported by gel analysis (Fig. 3).

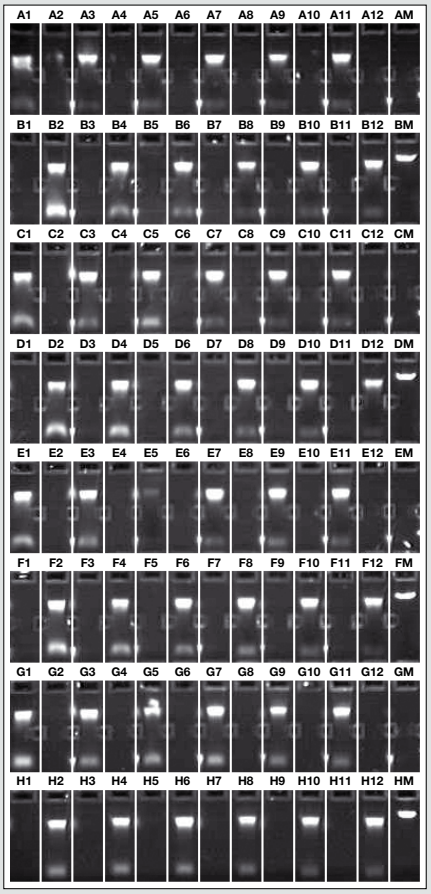

Fig. 3: *Evaluation of cross contamination by agarose gel electrophoresis of purified DNA samples from cabbage leaves processed in alternative wells.*

The wells AM, CM, EM and GM are the negative buffer control, BM, DM, FM and HM are the Lambda DNA positive control. RNA contamination is evident in some gels.

This may be avoided with a longer incubation with RNase A and bead mixture or increased levels of RNase A.

Conclusion

The integration of the Nucleon Plant DNA Kit with the ep*Motion* 5075 TMX platform provides a reliable, convenient and flexible system for the automated purification of high quality, high yield DNA from up to 96 plant samples in approximately 4 hours with minimal hands on time.

This easy to handle system benefits not only from increased throughput, but also reproducible purification of high quality, high molecular weight DNA for use in sensitive downstream applications.

Literature

Application Note 230: Automated Plant DNA Purification using the Nucleon® Plant DNA Kit on the ep*Motion*® 5075 TMX from Eppendorf (www.eppendorf.com/applications)

Nucleon Plant DNA Kit user manual (www.gen-probe.com/pdf)

Operation Manual for ep*Motion*® 5075 (www.eppendorf.com – Support & Services/ Literature Library/Manuals)

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News

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Some things are timeless: ever present, yet no one thinks about them. Like the Eppendorf Reference pipette, which has been on the market since 1994; or the Comforpette, its predecessor from the 1970s. Both are "one-button pipettes" with combined control button and ejector.

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"I didn't know any other pipettes. At my position at the Bogenhausen Hospital in Munich, I only worked with my Comforpette", says Kornelia Ewald. Responsible for conducting end user seminars on Liquid Handling at the Eppendorf Training Center, Ms. Ewald has worked at Eppendorf as Application Specialist since the beginning of the 1990s. As one-button pipettes continue to enjoy immense popularity, they are, of course, an integral part of the trainings.

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Dependable, safe, ergonomic

The Reference's continuous success is largely due to its unique design and technology concept. With its control button and ejector combination, the pipette is designed to actively reduce contamination from aerosols. All this is complemented by a high level of ergonomics: the pipette has only one control button and the volume can be easily read regardless of the position of the hand.

The Reference not only meets the high criteria of the Eppendorf PhysioCare Concept, its user friendliness has also been certified by the renowned TÜV Rheinland (Germany).

Positive feedback from countless users confirms: The Eppendorf Reference continues to be the reference class for modern liquid handling tools. With the utmost precision and accuracy, low failure rate and high longevity, it continues to please old and new fans, maintaining its strong position in the Eppendorf pipette portfolio.

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Tip

Nano Is All Around

Nanotechnology and Biotechnology explained clearly

Have you ever wondered about the early beginnings of biotechnology? How transgenic animals are created? Which technology tools are required? How two cells become one, and how this cell is then used as a "workhorse" in cancer research?

These and many more questions are answered by "Nano- and Biotechnology", an impressive permanent exhibit at the Centre for New Technologies of the "Deutsches Museum" in Munich, Germany, opened in November 2009.

For the explanation of the fascinating possibilities and developments within the life sciences, the scientific director of the exhibit, Dr. Sabine Gerber, places high value on realistic presentations which are easy to understand.

Several areas of the exhibit cover the "nanosystem cell" and the targeted alteration (re-programming) of these cells in order to employ them in the treatment of disease or the production of pharmaceuticals.

These methods are demonstrated in part using original Eppendorf cell technology products, specifically the Multiporator and a complete micromanipulation work station. Both these instruments have become the established standard equipment in scientific laboratories.

More information about the exhibit can be found at http://www.deutsches-museum.de/en/exhibitions/new-technologies/nanotechnology

More information about the topic cell technology may be found at www.eppendorf.com/cell

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Learning with Eppendorf Products

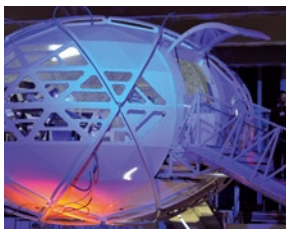
DNA Learning Centers in the USA

For more than twenty years, Eppendorf has been supporting the Dolan DNA Learning Center (DNALC), the world's first science center devoted entirely to genetics education and an operating unit of Cold Spring Harbor Laboratory located on Long Island, New York.

This support continues through Eppendorf North America ("ENA"), as the DNALC adds its third and youngest facility, designed to serve Manhattans inner city in Harlem, NY, USA. The Harlem DNA Lab was equipped with a wide range of Eppendorf laboratory products. "This included almost 100 pipettes, four microcentrifuges, several cyclers, and a variety of pipette tips and Eppendorf tubes", reports Dan Decker, Vice President of Sales for ENA and a member of the DNALC corporate advisory board.

The Harlem DNA Lab is a cooperation of the New York City Department of Education and the DOLAN DNA Learning Center/ Cold Spring Harbor Laboratory. In the newly created laboratory, school groups from New York City and the surrounding areas cover broad topics within the field of genetics. An extensive continuing education program is also offered for teachers. Since the founding of the DNA Learning Centers in 1988, more than 325,000 students and 8,000 teachers have profited from their program – naturally using Eppendorf products.

DNA Visitors' Laboratory at Deutsches Museum, Munich



Eppendorf also welcomed the opportunity of donating products like pipettes, thermomixers and centrifuges, to the DNA Visitors' Lab at the New Technologies Centre (ZNT) of the Deutsches Museum in Munich, Germany.

Assisted by young scientists, visitors can do their own experiments in the DNA Visitors' Laboratory, coming to grips with a pipette and other equipment used in the field of molecular biology. That helps them to understand the day-to-day work of a researcher in the lab while gaining useful knowledge on cell biology, heredity, and genetic engineering. For example, participants can use genetic fingerprints to solve a fictitious crime by processing DNA samples from the scene and those of a suspect and then comparing them.

The program is intended for interested participants in the 9th class or higher and is also offered as a teacher training session.

From the course program:

- Whodunnit? (Genetic fingerprinting with PCR)
- Genetically modified? (Genetic analysis with PCR)
- Genetic engineering activities (inserting a gene into a plasmid)

Understanding genetic research: Course participant at DNA Learning Center Harlem, New York, USA. More information at www.dnalc.org (Photo: DNALC Harlem)

Course participants in the DNA Visitors' Laboratory at Deutsches Museum. More informationen at www.deutsches-museum.de/en/exhibitions/new-technologies. (Photo: Deutsches Museum)

BERRIT HOFF & CAROLYN POWELL, EPPENDORF AG

Eppendorf Honors Young Scientists

Eppendorf Award for Young European Investigators 2011

On 25 May 2011, the *Eppendorf Award for Young European Investigators* which honors outstanding work in biomedical research in Europe was presented for the 16th time. For the first time, the award ceremony took place at the EMBL Advanced Training Centre in Heidelberg, Germany.

The 15,000 € prize went to Assistant Professor Suzan Rooijakkers (University Medical Center Utrecht, Depart. Medical Microbiology, Utrecht, The Netherlands), for her discoveries of how the pathogen *Staphylococcus aureus* evades immune attack to survive in the human host. She found out that the *Staphylococci* secrete proteins that block critical steps in the complement cascade. One such protein is the unique complement inhibitor SCIN that she found to inhibit the C3 convertase, which is required for the complement system to tag the bacteria for destruction. These results are creating new inroads into developing drugs against inflammatory and infectious diseases.

The *Eppendorf Young Investigator Award* is presented in partnership with *Nature*.

More information at www.eppendorf.com/award

Eppendorf & Science Prize for Neurobiology 2010

Congratulations to Canadian scientist, Dr. Christopher Gregg, Postdoctoral Fellow at Harvard University on winning the 2010 *Eppendorf & Science Prize for Neurobiology* for his research on maternal and paternal gene expression in the brain. Dr. Gregg's work focuses on genes that alter their expression in the brains of offspring according to whether they were inherited from the father versus the mother. Understanding the nature of parental effects on gene expression is potentially important for uncovering the basis of complex human neurological diseases such as autism and schizophrenia as well as eating disorders.

The annual international US$ 25,000 *Eppendorf & Science Prize for Neurobiology* honors young scientists for their outstanding contributions to neurobiological research. Dr. Gregg is the ninth recipient of this prestigious award. He was honored at a ceremony held on 15 November 2010 in San Diego, USA.

More information at www.eppendorf.com/prize

Tip

Applications Rewarded

Got a great application? Do you have an innovative application enhanced by Eppendorf products? Then tell us about it. If your application is found to be suitable, we will do our best to have it published either on our website, in an upcoming issue of Eppendorf BioNews or in a scientific journal.

Get a great reward! In case of publication, you will get 100 ep-points as well as free Eppendorf products worth up to € 500. For detailed guidelines about contents and format please visit www.eppendorf.com/applications.

If you have further questions please contact our Application Support colleagues by e-mail to support@eppendorf.com or give them a call at 0049 1803 666789.

AXEL JAHNS, EPPENDORF AG

Success through Competence

Sustained sales growth, Asia gaining in significance and an operating profit that was increased by 20.1 percent, all this characterizes Eppendorf's continued successful business performance in 2010.

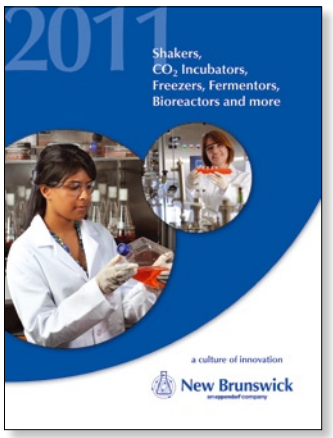
"After the upheaval of the economic crisis of 2009 the economy was not yet back on solid footing in 2010. In spite of this, Eppendorf can look back on a successful year based on strong growth in Asia and the recovery of the European and US economies", comments Klaus Fink, CEO of Eppendorf AG who swapped the Chair of the Executive Board with the Chair of the Supervisory Board on May 1, 2011. On the same day, Dr. Dirk Ehlers succeeded him as CEO.

Eppendorf's success has many good reasons

The excellent image in the life science industry, the customers, the product quality and the competence of the employees: these four pillars of Eppendorf's continued success are examined in more detail in the 2010 Annual Report. Order your personal copy now using the reply fax form on page 15, or check it out on our website.

New Brunswick Catalog 2011

New Brunswick Scientific joined the Eppendorf group in 2007, enriching the portfolio with a broad spectrum of innovative equipment for culture growth, detection and storage. Known for dependable operation, year after year, New Brunswick's shaker, CO_2 incubators, ultra-low temperature freezers, fermentors and bioreactors are used in a wide range of in research and commercial applications. New Brunswick Scientific's 2011 catalog is packed with innovative new equipment in virtually every product line.
You can order it by using the fax form on page 15!



News

Installation and Operational Qualification

Installation Qualification (IQ) and Operational Qualification (OQ) services assure that your Eppendorf instrumentation is delivered, installed and running according to manufacturer specifications. With the Installation Qualification we document that all items have been delivered and installed as specified. Operational Qualification uses the manufacturer's specified procedures and equipment for testing process critical measured values to ensure that your instruments are in a safe and optimal operating condition.

Eppendorf certification documents include relevant data conforming to Government and other regulatory internal laboratory requirements (FDA, ISO, GxP, SOP, etc.). They are supplied to help you prepare for your quality and regulatory documentation and audits.

Your value:

- Ensures the safety and quality of your work
- Proof of compliance with the relevant technical specifications
- Support for your regulatory tasks and GxP requirements

epServices
for premium performance

Contact your local Eppendorf office for more information or visit www.eppendorf.com/epservices and/or your local Eppendorf website.

Prize Competition

Win online at www.eppendorf.com/bn-service

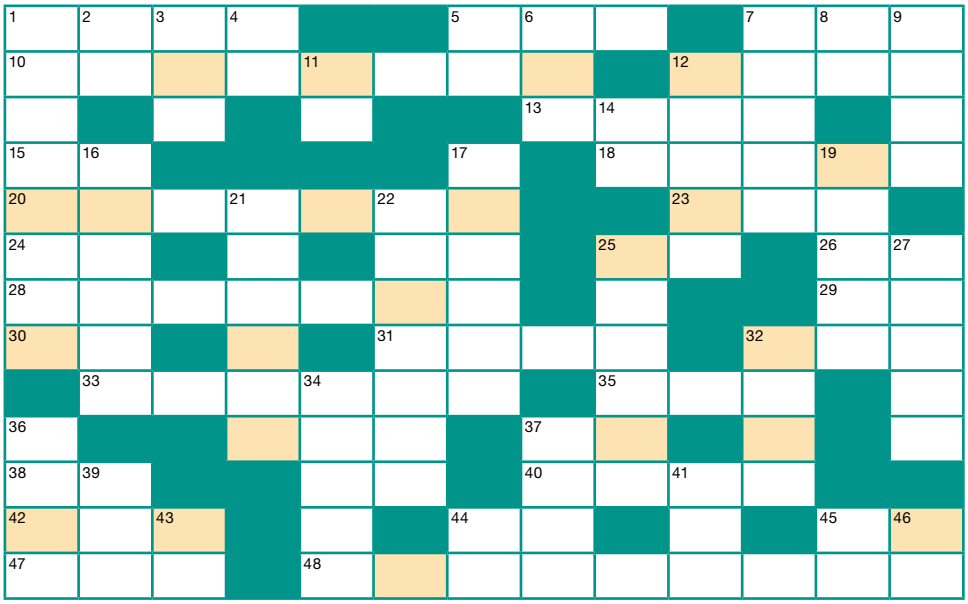
The solution of the prize competition of BioNews No. 33 was "Eppendorf LoBind". Claudia Bräunig (fzmb GmbH, Bad Langensalza, Germany) won the first prize, an Eppendorf Xplorer pipette.

Have fun in our new crossword!

How to find out the solution: Simply arrange the encircled letters of the crossword in the correct order. Send us the solution until **31st October 2011**.

You can either use the reply fax (p.15), send us an e-mail to bionews@eppendorf.de, or participate online at www.eppendorf.com/bn-service.

All correct answers will be considered for a prize. Winners will be notified in writing. Cash payment of the prize is not possible. No recourse to legal action. The judges' decision is final. Eppendorf employees and their families may not participate. The winner of the first prize will be published in BioNews No. 37.



1st prize:

1 epReference³ Pack

including 3 Eppendorf Reference pipettes (0.5–10 µL, 10–100 µL, 100–1,000 µL), matching epT.I.P.S., 3 pipette holders and a pipette pen.

2nd to 5th prize:

1 MP3 player each

6th to 15th prize:

200 bonus ep-points each

ACROSS

1 Opposite of cold
5 Pub, tavern
7 Crowd, pack, gang – comes in a flash, nowadays
10 The first of its kind, no fake
12 Complements or completes gamy, poly and tony
13 Its plural would be "those"
15 1000 metres (abbr.)
18 Measures and indicates the time
20 If this is good you feel comfortable
23 Something John Volta is missing
24 High definition, high density (abbr.)
25 This US state (abbr.) has Harrisburg as its capital
26 State on the West coast of the USA (abbr.)
28 City in 26 across
29 High throughput (abbr.)
30 ISO country code of Pakistan
31 Business, contract, bargain
32 French salt
33 French future
35 Loud laughter on the web
37 Marking that confirms a product's compliance with EU legislation
38 Chemical symbol for aluminum
40 Smallest particle of an element
42 Famous cape in Massachussetts, USA
44 Internet Protocol (abbr.)
45 Postscript
47 I in Latin
48 Popular one-button pipette

DOWN

1 Room or building with tools or machinery
2 Chemical symbol for argon
3 Spanish word for river
4 Chemical symbol for magnesium
5 Barium (abbr.)
6 Special computer key
7 Drive, engine
8 Ontario (abbr.)
9 Piece of literature
11 Postal abbreviation for Illinois
12 Southern European country in the Mediterranean Sea
14 Honoris causa (abbr.)
16 Japanese rice fish
17 Scanning device (e.g., for bar codes or cards)
19 Stores data
21 Geographic term
22 Genus of yeast
25 Visible result of centrifugation
27 Collection of maps
32 Slender, thin
34 The direction opposite of the zenith
36 Front part of the head
37 Poncho, sleeveless garment
39 Logarithm (abbr.)
41 Lyrical verse
43 Perform, carry out, execute
44 In the event that (conj.)
45 Personal computer (abbr.)
46 ISO country code of Sweden

To Eppendorf AG, Hamburg, Germany, Attn. Anton Janott, Fax (+49) 40 - 5 38 01-8 40

(Please – print or type, no private address)

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○	223	real-time PCR Consumables	***I have comments on the BioNews No. 35:***	
○	224	Mastercycler® pro		
○	228	ep*Motion*® 5075 TMX		
○	235	Centrifuge 5424 R		
○	Eppendorf General Catalog 2011		***I have suggestions/topics for the next BioNews:***	
○	Eppendorf Annual Report 2010			
○	New Brunswick Catalog 2011			

Solution of BioNews No. 35 prize competition:

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Closing date: 31st October 2011 (Visit www.eppendorf.com/bn-service to participate online!)